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(54) PROCEDE DE DOSAGE D'UNE SUBSTANCE IMMUNOLOGIQUE AU MOYEN DE PARTICULES DE LATEX MAGNETIQUES ET DE PARTICULES NON-MAGNETIQUES

VERFAHREN ZUR BESTIMMUNG EINER IMMUNOLOGISCHEN AKTIVEN SUBSTANZ MITTELS MAGNETISCHER LATEXTEILCHEN UND NICHTMAGNETISCHER TEILCHEN

METHOD FOR ASSAYING AN IMMUNOLOGICAL SUBSTANCE USING MAGNETIC LATEX PARTICLES AND NON-MAGNETIC PARTICLES

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Description

Domaine de l'invention

[0001] La présente invention a trait à un nouveau procédé de dosage d'une substance immunologique choisie parmi l'ensemble constitué par les antigènes et les anticorps, ledit procédé mettant en oeuvre des particules de latex magnétiques servant de "support" à la réaction immunologique

antigène + anticorps → antigène-anticorps

et des particules non-magnétiques servant de "marqueur" dans ladite réaction.

[0002] Elle concerne également, en tant que produits industriels nouveaux, les trousses ou nécessaires de dosage comprenant lesdites particules magnétiques et non-magnétiques sensibilisées avec des composants (ou substances) immunologiques.

Art antérieur

[0003] Historiquement, on sait que des techniques de dosage reposant sur l'agglutination macroscopique (i.e. agglutination visible à l'oeil nu) de particules de latex sensibilisées par un antigène (ou respectivement un anticorps) par mise en contact avec un conjugué anticorps (ou respectivement antigène) ont été présentées pour la première fois dans l'article de J.M. SINGER, American Journal of Medecine, 31, pages 766-779, (1961).

[0004] Ces techniques de dosage ont été améliorées, en ce qui concerne la sensibilité et la durée de réaction, en remplaçant la détection à l'échelle macroscopique de l'agglutination, par une détection à l'échelle microscopique de la réaction d'agglutination antigène/anticorps au moyen d'une variation de l'intensité lumineuse transmise ou diffractée (i.e. diffusée). Ainsi ont été proposés des dosages quantitatifs ou qualitatifs par turbidimétrie ou par néphélométrie.

[0005] Dans le domaine de la turbidimétrie (par mesure de l'absorbance de la lumière), on connaît des articles de A.M. BERNARD et al., Clin. Chem., 27 (No. 6), pages 832-837, (1981) et de A.M. BERNARD et R.R. LAUWERYS, Clin. Chem. Acta, 119, pages 335-339, (1982), l'utilisation de particules submicroniques de latex (diamètre moyen : 0,79 micromètre) revêtues d'anticorps anti (β_2-microglobuline) par mesure de l'absorbance à 360 nm ; de l'article de Y. MAYNARD et al., Clin. Chem., 32 (No. 5), pages 752-757, (1986), l'utilisation d'anticorps monoclonaux ou polyclonaux anti-IgG pour le dosage, dans les microcuvettes, d'immunoglobulines sériques.

[0006] Dans le domaine de la néphélométrie (par mesure de la diffusion de la lumière) on connaît de l'article de J. GRANGE et al., Journal of Immunological Methods, 18, pages 365-375, (1977) l'utilisation de billes de latex submicroniques (diamètre moyen : 0,3 micromètre) revêtues d'un anticorps anti-IgG de lapin, pour le dosage d'IgG de lapin. Les billes de latex sont ici constituées par du polystyrène carboxylé contenant les groupes COOH pour fixer le ligand ou composant immunologique, dans le cas d'espèce l'anticorps anti-IgG de lapin. Cet article signale notamment que "la préparation de sphères de latex contenant des antigènes ou des anticorps adsorbés est difficile à standardiser en raison de leur tendance à s'auto-agglutiner lorsque le pH et la force ionique du milieu de dosage changent"

[0007] On connaît également de l'article d'Anne-Marie BONNEFOY et al., C.R. Acad. Sc. Paris, 283, série D, pages 115-118 (5 juillet 1976) l'utilisation de sphères de latex (polystyrène) d'un diamètre de 300 nm liées par covalence à un anticorps pour la réalisation de dosages néphélométriques (lecture sous une longueur d'onde de 550 nm, selon un angle d'observation de 90°).

[0008] En bref, les méthodes turbidimétriques et néphélométriques, qui sont utilisables pour des concentrations de substance immunologique supérieures à 0,1 mg/ml (méthodes turbidimétriques) ou supérieures à 0,02 mg/ml (méthodes néphélométriques), requièrent un matériel très "spécialisé" et un traitement préalable des échantillons. Elles sont limitées par plusieurs inconvénients à savoir :

- difficultés à réaliser des suspensions stables et standardisées,
- auto-agglutination spontanée des latex sensibilisés par un anticorps ou un antigène,
- faible plage de mesure de la courbe d'étalonnage (variation de la densité optique (OD) n'excédant pas 0.3 à 0.4),
- manque de sensibilité et de reproductibilité,
- traitement du réactif particulaire avant emploi dans certains cas (pour désagrégation), et mauvaise stabilité du réactif,
- mise en oeuvre délicate et détection parfois complexe.

Ces inconvénients ont fait que ces méthodes n'ont pas été exploitées malgré leur intérêt pratique.

[0009] On connaît par ailleurs de US-A-5 175 112 (J. AMIRAL et al.) un procédé de dosage turbidimétrique ou néphélométrique par agglutination d'un réactif constitué par des particules de latex submicroniques sensibilisées par un anticorps (ou respectivement un antigène) en présence de l'antigène correspondant à tester, ce procédé permettant d'améliorer la sensibilité (seuil de sensibilité de l'ordre de 100 ng/ml par turbidimétrie, et de l'ordre de 10 ng/ml par néphélométrie) par un choix particulier de la nature du latex, de la granulométrie des particules dudit latex, du mode de stabilisation desdites particules, de la force ionique et du pH du milieu réactionnel liquide.

[0010] Par ailleurs, on connaît des trousses ou nécessaires de dosage comprenant des particules de latex

magnétiques et plus particulièrement des particules de latex paramagnétiques (les fabricants de particules de latex les appellent notamment des particules de latex "superparamagnétiques"). Il se trouve que les protocoles de dosage fournis avec ces trousses (notamment celui des trousses commercialisées par les sociétés dites AMERSHAM et CIBA-CORNING) mettent en oeuvre un procédé différent de celui de l'invention, en ce sens que lesdites particules de latex magnétiques sensibilisées par un matériau immunologique interviennent principalement pour remplacer, dans une méthode EIA ("enzymoimmunoassay") ou RIA ("radioimmunoassay"), une opération de filtration ou centrifugation. En particulier les protocoles de dosage des trousses connues contenant des particules de latex magnétiques (i) ne concernent pas le domaine de l'agglutination, et (ni) ne décrivent ni ne suggèrent en particulier le procédé préféré selon l'invention dit de dosage "en retour" direct ou en un seul temps (c'est-à-dire en opérant dans le même milieu et le même récipient de réaction, sans rinçage ni apport supplémentaire de réactif ou diluant), d'une part, ni le dosage "direct" (inverse du dosage dit en "retour") par inhibition compétitive en un seul temps, d'autre part.

[0011] Par ailleurs, on connaît de US-A-4 115 535 (I. GIAEVER), JP-A-5 180 842 (NIPPON PAINT Co. Ltd.) [résumé dans DATABASE WPI de DERWENT, No. 93-267053 et publié le 23.07.1993] et de WO-A-8606493 (LABSYSTEMS OY) une technique de dosage/identification selon laquelle on fait

(1) réagir (a) des particules sensibilisées par le même antigène, ces particules étant les unes magnétiques les autres non-magnétiques et colorées, avec (b) un échantillon susceptible de contenir un anticorps spécifique dudit antigène, et
(2) appliquer un champ magnétique pour observer dans la portion non-agglutinée du milieu réactionnel liquide, une diminution de la densité optique traduisant la présence dudit anticorps dans ledit échantillon.

[0012] Selon le résumé de JP-A-5 180 842 précité, on peut également faire réagir (a) des particules magnétiques et non-magnétiques sensibilisées par le même anticorps avec (b) un échantillon susceptible de contenir l'antigène correspondant.

[0013] Cette technique de compétition/agglutination présente cependant l'inconvénient de ne pas être suffisamment sensible par rapport aux méthodes EIA et RIA connues.

[0014] On connaît enfin de EP-A-0 410 893 (MITSUBISHI KASEI) une technique de dosage (ou identification) d'un anticorps susceptible d'être présent dans un échantillon de fluide biologique, selon laquelle

(1) on fait réagir ledit échantillon avec un mélange comprenant (a) des particules non-magnétiques sensibilisées par l'antigène spécifique dudit anticorps, et (b) des particules magnétiques sensibilisées par un produit fixant les anticorps [tel que la protéine A, une autre substance fixant le fragment Fc des anticorps ou un anti(anticorps)],
(2) on applique un champ magnétique pour séparer les particules non-magnétiques qui n'ont pas été agglutinées avec les particules magnétiques, et
(3) on évalue la quantité de particules non-magnétiques qui n'ont pas été agglutinées pour en déduire la quantité d'anticorps présente dans ledit échantillon.

[0015] Dans cette dernière technique, les particules magnétiques sensibilisées par le produit fixant les anticorps interviennent comme un moyen amplificateur de la réaction de l'antigène avec l'anticorps que l'on veut déterminer. Cependant cette technique présente l'inconvénient de ne pas être suffisamment sensible par rapport aux méthodes EIA et RIA connues.

But de l'invention

[0016] Un des buts de l'invention est de proposer une nouvelle solution technique pour le dosage, la détermination ou l'identification par agglutination à l'échelle microscopique, qui soit essentiellement plus rapide et subsidiairement plus sensible que les méthodes antérieurement connues de dosage d'agglutination par turbidimétrie, néphélométrie ou champ magnétique.

[0017] L'objectif idéal, en ce qui concerne la sensibilité, serait d'atteindre, par une technique d'agglutination ou par une technique homogène en un seul temps, ne nécessitant pas les lavages habituels des techniques EIA, le seuil (concentration minimale de la substance immunologique à doser se situant dans la gamme de 10 ng/ml à 1 ng/ml) des techniques EIA ou RIA.

[0018] Un autre but de l'invention est de pallier aux inconvénients précités des techniques de dosage de l'agglutination par turbidimétrie, néphélométrie ou champ magnétique.

[0019] Selon l'invention est envisagée pour le dosage ou l'identification d'une substance immunologique [X], une nouvelle solution technique mettant en oeuvre des particules magnétiques et non-magnétiques qui sont (i) les unes sensibilisées par un anticorps [antiX] de ladite substance immunologique, et (ni) les autres sensibilisées par ladite substance immunologique [X], un anticorps [anti(antiX)] dudit anticorps [antiX] ou un anticorps identique ou différent dudit anticorps [antiX].

Objet de l'invention

[0020] Ces buts sont atteints grâce à la nouvelle solution technique selon l'invention permettant de détecter la portion non agglutinée (avec un photomètre de laboratoire relativement simple) d'un système comprenant un réactif immunologique constitué par des particules de latex magnétiques sensibilisées par un premier com-

NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3, 5% goat serum and 1% fish gelatin at pH 7.2.

d. Immunoassay protocol

The indicator reagent (140 µl) was mixed with a series of samples (50 µl) containing known amounts of hCG in normal human serum. The mixtures were incubated for 10 minutes at 31-32°C. The anti-hCG antibody-PGA capture reagent (100 µl) was added, and the reaction mixtures were incubated for 10 minutes. An aliquot of each reaction mixture (200 µl) was applied to the solid phase material, followed by a wash. An enzyme substrate (70 µl; as described in Example 9) was added, and the resulting rate of fluorescence was measured. The results of the assay are shown in Table 16. The results demonstrate that as the hCG test sample concentration increased there was a corresponding increase in the formation of capture reagent/analyte/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased.

TABLE 16
hCG Ion-capture Sandwich Assay
Capture reagent: anti-hCG antibody-PGA
Indicator reagent: alkaline phosphatase-labeled anti-hCG antibody

	Rate of fluorescence (counts/sec/sec) hCG-specific capture reagents
hCG (mIU/ml)	hCG-ITC-PGA
0	22
8	38
40	116
100	236
550	644
200,000	2058

Example 14

Ion-capture Flow-Through Device for a Two-Step hCG Assay

a. Preparation of the solid phase

Test sample application pads (glass fiber matrix) were treated with various concentrations of an aqueous solution of Merquat-100® polymeric ammonium compound, 100 mM Tris, 100 mM sodium chloride, 0.1% fish

gelatin, 0.1% sucrose and 0.1% sodium azide. The application pads were allowed to dry, and the pads were overlaid upon a layer of absorbent material. Substantially the same procedure was used to prepare a flow-through solid phase device treated with Celquat® L-200 polymeric compound. Alternative devices were prepared by treating the application pad with Merquat-100® polymeric quaternary ammonium compound (a cationic homopolymer of dimethyldiallylammonium chloride, 0.5% in water) immediately before use.

b. Preparation of the indicator reagent

The indicator reagent was a conjugate of goat anti-β-hCG antibody and alkaline phosphatase, diluted in 1% Brij®-35 polyoxyethylene (23) lauryl ether (Sigma), 100 mM Tris, 500 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3 and 0.5% non-fat dry milk at pH 7.2. The indicator reagent was filtered through a 0.22 μm filter before use.

In alternative indicator reagent preparations, dextran sulfate (MW 5,000) or heparin was included as a nonspecific binding blocker. The blocker was used to enhance the signal-to-noise ratio by inhibiting the binding of the labeled antibody to non-analyte.

c. Preparation of the capture reagent

A monoclonal anti-β-hCG antibody-PGA capture reagent was prepared substantially in accordance with the method described in Example 8.c. above. Every five milliliters of the coupling reaction mixture was fractionated on a gel filtration chromatography column (2.4 x 54 cm, at a 0.4 ml/minute flow rate). The elution buffer contained 0.1 M sodium phosphate, 0.3 M NaCl and 0.05% NaN_3, at pH 8.5. The polymeric anion/antibody conjugate was diluted with 25 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3, 10% normal mouse serum and 1% fish gelatin at pH 7.2. The capture reagent was filtered through a 0.22 μm filter before use.

d. Immunoassay protocol

The capture reagent (80 μl) was mixed with an equal volume of test sample containing a known amount of hCG in normal human serum. The mixture was incubated at approximately 31-32°C for approximately twelve minutes. The specific binding reaction resulted in the formation of a capture reagent/analyte complex.

Each reaction mixture (80 µl) was then applied to a flow-through device, followed by a wash with Tris buffered saline (75 µl). The indicator reagent (50 µl) was then applied to the solid phase device and incubated for twelve minutes. The device was then washed two times.

An enzyme substrate (70 µl; 1.2 mM 4-methylumbelliferyl-phosphate in a solution of 100 mM AMP, 0.01% EDTA, 0.1% NaN_3, and 4.0 mM tetramisole at pH 10.3) was added, and the resulting rate of fluorescence was measured. The results of the assay are shown in Tables 17-19. The results demonstrated that as the hCG test sample concentration increased there was a corresponding increase in the formation of capture reagent/analyte/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased. The results show that the signal to noise ratio is improved by including a nonspecific binding blocker in the indicator reagent. Furthermore, the results demonstrated that the cationic homopolymer of dimethyldiallylammonium chloride was a preferred polymeric cation for the preparation of the solid phase for use in two-step assays wherein the device is subjected to one or more washings, e.g., the Merquat-100® polymeric ammonium compound has a nitrogen content of about 10% (exclusive of counter ion), whereas the Celquat® H-100 polymeric compound has a nitrogen content of about 1% (exclusive of counter ion).

TABLE 17

hCG Ion-capture two-step Sandwich Assay
Capture reagent: anti-β-hCG antibody-PGA (0.5 µg/test)
Indicator reagent: alkaline phosphatase-labeled anti-β-hCG antibody (with and without nonspecific binding blocker)
Solid phase: coated with a cationic homopolymer of dimethyldiallylammonium chloride immediately before use

	Rate of fluorescence (counts/sec/sec)	
hCG (mIU/ml)	2% dextran sulfate	no blocker
0	68	255
100	1028	1104

TABLE 18

hCG Ion-capture two-step Sandwich Assay

Capture reagent: anti-β-hCG antibody-PGA (0.5 µg/test)

Indicator reagent: alkaline phosphatase-labeled anti-β-hCG antibody (with blocker)

Solid phase: with varying cationic polymer concentration

	Rate of fluorescence (counts/sec/sec) Merquat-100® polymeric ammonium compound (%w/v)				
hCG (mIU/ml)	0.02	0.04	0.2	0.4	0.6
0	34	31	26	30	39
100	514	578	627	661	647

TABLE 19

hCG Ion-capture two-step Sandwich Assay

Capture reagent: anti-β-hCG antibody-PGA

Indicator reagent: alkaline phosphatase-labeled anti-β-hCG antibody

Solid phase: with 0.125% Celquat® H-100 polymeric compound

	Rate of fluorescence (counts/sec/sec) quantity of capture antibody (µg/test)		
hCG (mIU/ml)	0.268	0.402	0.652
0	86	100	115
100	186	202	259

Example 15

Ion-capture Flow-Through Device for Thyroid Stimulating Hormone (TSH) Assay

a. Preparation of the solid phase

An application pad (glass fiber matrix) was treated with an aqueous solution of Merquat-100® polymeric ammonium compound substantially in

accordance with the procedure described in Example 14.a. The pad was then overlaid upon a layer of absorbent material to complete the flow-through solid phase device.

b. Preparation of the indicator reagent

The indicator reagent was a conjugate of goat anti-β-hCG antibody and alkaline phosphatase, diluted in 1% Brij®-35 polyoxyethylene (23) lauryl ether, 1% fish gelatin, 100 mM Tris, 500 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3 and 0.5% non-fat dry milk at pH 7.2. The indicator reagent was filtered through a 0.22 µm filter before use. Dextran sulfate (0.5%, MW 5,000) was added as a nonspecific binding blocker.

c. Preparation of the capture reagent

The capture reagent was prepared by coupling a Protein A purified monoclonal anti-TSH antibody with carboxymethyl amylose (CMA; Polysciences, Inc., Warrington, PA). Coupling was performed using a water-soluble carbodiimide reagent (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDCI) substantially in accordance with the following procedure.

The coupling mixture contained an antibody solution (2 ml; 1 mg/ml in MES buffer [25 mM, 2-(N-Morpholino)ethanesulfonic acid] pH 5.5) and CMA (1.6 ml; 10 mg/ml in MES buffer). To the solution was added, with stirring, a freshly prepared EDCI solution (40 µl; 100 mg/ml in MES buffer). The reaction mixture was stirred at room temperature for 40 minutes. The reaction was quenched by adding a 25 % glycine solution (67 µl), and the product was then fractionated by gel filtration chromatography using a TSKgel G4000SW column (2.15 cm x 30 cm) fitted with a TSKguard column SW (2.15 cm x 7.5 cm; Anspec Co., Ann Arbor, Michigan). The column was eluted with PBS (0.1 M sodium phosphate, 0.3 M NaCl and 0.05% sodium azide, at pH 6.8). The purified Antibody/CMA capture reagent was diluted in a diluent containing 50 mM Tris, 300 mM NaCl, 1% bovine serum albumin, 2.5% fish gelatin and 0.1% NaN_3, at pH 7.5.

d. Immunoassay protocol

The capture reagent (30 µl) and Tris buffered saline (100 µl; 500 mM Tris, 300 mM NaCl and 0.1% NaH_3) were mixed with a test sample (50 µl) containing a known amount of hCG in normal human serum. The reaction mixture was incubated at approximately 33-34°C for approximately ten minutes. The

specific binding reaction resulted in the formation of a capture reagent/analyte complex.

An aliquot of each reaction mixture (140 µl) was applied to a solid phase device, followed by a wash with Tris buffered saline (150 µl). The indicator reagent (70 µl) was applied to the device and incubated for approximately ten minutes. The device was then washed two times with buffer (100 µl each). The enzyme substrate (70 µl; 1.2 mM 4-methylumbelliferyl-phosphate in a solution of 100 mM AMP, 0.01% EDTA, 0.1% NaN_3, and 4.0 mM tetramisole at pH 10.3) was added, and the resulting rate of fluorescence was measured.

The results of the assay are shown in Table 20. The results demonstrated that as the concentration of TSH in the test sample increased, there was a corresponding increase in the formation of capture reagent/analyte/indicator reagent complex. Therefore, the amount of detectable label associated with the solid phase increased as the concentration of analyte increased. The results also demonstrated that the combination of Merquat-100® polymeric ammonium compound with polyacrylic acid or with carboxymethylamylose provided a solid phase and capture reagents which were advantageously used in two-step assays wherein the device is subjected to one or more washings or manipulations.

TABLE 20
TSH Ion-capture Two-step Sandwich Assay
(using polyacrylic acid or carboxymethylamylose polyanions)

	Rate of fluorescence (counts/sec/sec)	
TSH (mIU/ml)	carboxymethylamylose	polyacrylic acid
0	7.1	6.4
0.5	13.3	12.1
2.0	34.7	28.7
10.0	147.5	119.8
40.0	513.9	442.6
100.0	1121.6	995.5

e. TSH capturing efficiency

Radioiodinated TSH was used in the assay protocol, as described in Example 15. d, to demonstrate the more efficient TSH capturing of CMA-coupled antibodies

than that of polyaspartic- and polyglutamic-coupled antibodies. The coupling of antibodies to the polyanions was performed substantially in accordance with the method described above (Example 15.c.) After the rate of fluorescence was measured at the end of the assay protocol, the radioactivity of TSH captured on the solid phase material was also measured by means of a scintillation spectrometer (Auto-Logic, Abbott Laboratories, North Chicago, IL). The results of this procedure are demonstrated in Table 20 (a).

TABLE 20 (a)

Capture of Radiolabeled TSH in the Cationic Solid Phase Material

Polyanion-coupled anti-TSH antibody	%TSH captured	Rate of fluorescence (counts/sec/sec)
Carboxymethylamylose	70	662
Polyaspartic Acid	1.5	37
Polyglutamic Acid	2.0	57

Example 16

Ion-capture Flow-Through Device for a One-Step hCG Assay

a. Preparation of the solid phase

A glass fiber matrix was treated with an aqueous solution of Merquat-100® polymeric ammonium compound substantially in accordance with the procedure described in Example 14. a, above. The pad was then overlaid upon a layer of absorbent material to complete the device.

b. Preparation of the indicator reagent

The indicator reagent was a goat anti-β-hCG antibody conjugated to alkaline phosphatase and diluted in 3.33% Brij®-35 polyoxyethylene (23) lauryl ether, 5 mM Tris, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3 and 5% fish gelatin at pH 7.2. The indicator reagent was filtered through a 0.2 μm filter before use. In alternative indicator reagent preparations, carboxymethyl cellulose (MW 250,000) or carboxymethyl dextran was included as a nonspecific binding blocker.

c. Preparation of the capture reagent

A monoclonal anti-hCG antibody-PGA capture reagent was prepared substantially in accordance with the method described in Example 15. c, above. The polymeric anion/antibody conjugate was diluted with 3.33% Brij®-35 polyoxyethylene (23) lauryl ether, 5 mM Tris, 500 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3, and 5% fish gelatin at pH 7.2. The enzyme substrate was 1.2 mM 4-methylumbelliferyl-phosphate in a solution of 100 mM AMP, 0.01% EDTA, 0.1% NaN_3, and 4.0 mM tetramisole at pH 10.3.

d. Immunoassay protocol

The capture reagent (50 µl), indicator reagent (55 µl) and sample diluent buffer (35 µl: 75% normal calf serum, 25% normal goat serum and 0.2% NaN_3, filtered through a 0.22 µm filter before use) were mixed with a test sample (30 µl) containing a known amount of hCG in normal human serum. The mixture was incubated at approximately 33-34°C for approximately fourteen minutes. The specific binding reaction resulted in the formation of a capture reagent/analyte/indicator reagent complex.

An aliquot of each reaction mixture (110 µl) was then applied to a solid phase device, followed by two washes with Tris buffered saline (75 µl). The enzyme substrate (65 µl) was added, and the resulting rate of fluorescence was measured.

The results of the assay are shown in Table 20. The results demonstrated that as the hCG test sample concentration increased there was a corresponding increase in the formation of capture reagent/analyte/indicator reagent complex, and therefore, the amount of detectable label associated with the solid phase increased. Furthermore, the results show that the signal to noise ratio was improved when a free polyanionic substance was included in the indicator reagent as a nonspecific binding blocker, even though the capture reagent was a polymeric anion/antibody conjugate.

TABLE 21

hCG Ion-capture Sandwich Assay

Capture reagent: anti-hCG antibody-PGA

Indicator reagent: alkaline phosphatase-labeled anti-hCG antibody

	Rate of fluorescence (counts/sec/sec) % carboxymethyl cellulose in indicator reagent			
hCG (mIU/ml)	0	0.01	0.25	0.5
0	37.2	23.8	17.2	13.3
10	76.8	58.4	48.8	42.1
1000	1803.6	1665.4	1692.2	1507.2

	Rate of fluorescence (counts/sec/sec) % carboxymethyl dextran in indicator reagent			
hCG (mIU/ml)	0	0.01	0.25	0.5
0	35.6	30.0	17.8	14.8
10	75.2	68.4	54.7	49.8
1000	1826.6	1851.2	1739.5	1646.6

Example 17

Ion-Capture Teststrip for an hCG Sandwich Assay

a. Preparation of the solid phase

A rectangular zone on a central portion of a strip of nitrocellulose (5 µm pore size; Schleicher & Schuell; Dassel, Germany) was treated with an aqueous solution of 0.05% Merquat-100® polymeric ammonium compound and 10 mM Tris to form a positively charged capture or detection zone.

b. Preparation of the indicator reagent

The indicator reagent was made of colloidal selenium particles coated with mouse monoclonal anti-hCG antibody. The indicator reagent was appropriately diluted (as determined by titer curve) in assay buffer containing 50 mM Tris, 2% lactose, 2% casein, 1% goat serum and 1% mouse serum at pH 8.4.

c. Preparation of the capture reagent

A goat anti-β-hCG antibody was coupled to poly-L-glutamic acid using 1-ethyl-3-(3-dimethylaminopropyl)-carbodimide substantially in accordance with the method described in Example 15. c, above. The capture reagent was then appropriately diluted in the same diluent as the indicator reagent.

d. Immunoassay protocol

The indicator reagent (50 µl) was mixed with an equal volume of capture reagent. The mixture was then combined with a series of samples (0, 50, 100 and 250 mIU/ml; 150 µl each) containing known amounts of hCG in normal human urine. The resultant reaction mixtures were incubated for five minutes at room temperature. The specific binding reaction resulted in the formation of a capture reagent/analyte/indicator reagent complex.

Each reaction mixture (250 µl) was then applied to one end of the prepared strip of nitrocellulose. The mixture was allowed to migrate through the strip to the capture zone and through the zone. Capture reagent and complexes thereof were retained at the capture zone, wherein the indicator reagent complexed with the retained capture reagent indicated the amount of analyte in the test sample as well as the the presence of analyte in the test sample. The 0 mIU/ml test sample produced no coloration of the capture zone. The 50, 100 and 250 mIU/ml test samples produced visible coloration of the capture zone.

Example 18

Ion-Capture Teststrip Device for an hCG Assay

a. Preparation of the solid phase

A rectangular zone on a central portion of a strip of nitrocellulose (5 µm pore size; Schleicher & Schuell) was treated with an aqueous solution of 1% Celquat® L-200 polymeric compound to form a positively charged capture zone. The cationic polymer was dispensed using a #29 gauge tube (MICRO Inc., Elmhurst, NY) moving at a rate of 0.5 inches/second with a flow rate of 0.05 milliliter/minute.

b. Preparation of the indicator reagent

The indicator reagent was made of colloidal selenium particles coated with mouse monoclonal anti-hCG antibody. The indicator reagent was appropriately diluted (as determined by titer curve) in assay buffer containing 50 mM Tris, 2% lactose, 2% casein, 1% goat serum and 1% mouse serum at pH 8.4.

c. Preparation of the capture reagent

An anti-β-hCG antibody was coupled with poly-glutamic acid substantially in accordance with the method described in Example 8.c, above.

d. Preparation of the assay device

A reagent pad or test sample application pad was prepared by soaking a pad of absorbant material (40 µpore glass fiber material; Lydall Inc., Hamptonville, NC) with a mixture containing the capture reagent (20 µg/ml) and the indicator reagent (antibody concentration 0.024 mg/mL, selenium concentration 0.3 mg/mL) in Tris buffered saline (0.1 M Tris, 0.9% NaCl, pH 7.8), 1.0% casein. The pad was then air dried. The teststrip device was then constructed by contacting the test sample application pad and nitrocellulose strip, and then double laminating the pad and nitrocellulose so that the application pad overlapped at least an end portion of the nitrocellulose strip offset from the capture zone.

e. Immunoassay protocol

A test sample containing a known amount of hCG in normal human urine (0, 50 and 250 mIU/ml; 50 µl each) was applied to the test sample application pad of the assay device, or the application pad was dipped into the test sample. The test sample, resolubilized assay reagents and complexes thereof migrated from the application pad to and through the nitrocellulose strip. After five minutes, at room temperature, the specific binding reaction and the ion-capture reaction resulted in the formation of a capture reagent/analyte/indicator reagent complex which was immobilized at the capture zone of the teststrip. Unbound indicator reagent and test sample components passed through the capture zone. The 0 mIU/ml test sample produced no detectable signal at the capture zone. The 50 mIU/ml test sample produced a faintly detectable visible signal at the capture zone. The 250 mIU/ml test sample produced a strongly detectable visible signal at the capture zone. The assay results also demonstrated that a homogeneous specific binding reaction could form a tertiary complex while reacting in a solid phase teststrip device.

Example 19

Ion-Capture Teststrip Device for a Phenylcyclidine (PCP) Assay

a. Preparation of the solid phase

A rectangular zone on a central portion of an elongated strip of nitrocellulose (3 mm in width) was treated with an aqueous solution of 0.5% Merquat-100® polymeric ammonium compound to form a positively charged capture zone.

b. Preparation of the indicator reagent

The indicator reagent was made of colloidal selenium particles coated with PCP antibody.

c. Preparation of the capture reagent

A PCP antigen was conjugated to poly-glutamic acid substantially in accordance with the method described in Example 10. a, above.

d. Preparation of the assay device

An assay reagent pad (3 mm in width) or test sample application pad was prepared by soaking an absorbant material (Whatman PD075 glass fiber filter; Whatman Specialty Papers, Clifton, NJ) with the indicator reagent (2.5 mg/ml; 4% casein, 4% sucrose, 1% polyethylene glycol [MW 15,000-25,000] in 0.01 M Tris). The application pad was then air dried. The application pad and nitrocellulose where then assembled so that the reagent pad overlapped one end of the nitrocellulose by approximately one millimeter.

e. Immunoassay protocol

The capture reagent (15 µl) and an equal volume of test sample, containing a known dilution of PCP in distilled water (1:10, 1:100, 1:1000, 1:10000), were mixed. The mixture was applied to the test sample application pad. The mixture was allowed to migrate through the pad and strip for at least ten five minutes. The competitive binding reaction resulted in the formation of capture reagent/indicator reagent complex and indicator reagent/analyte complex, wherein the amount of capture reagent/indicator reagent complex decreased as the amount of analyte in the test sample increased. The

polyelectrolyte reaction resulted in the immobilization of the capture reagent/indicator reagent complex in the capture zone of the teststrip. Unbound indicator reagent and unreacted test sample components, as well as indicator reagent/analyte complex, passed through the capture zone. The assay results demonstrated that the higher the amount of PCP in the test sample, the lower the detectable signal at the capture zone. The assay results also demonstrated that a homogeneous specific binding reaction could take place in a solid phase teststrip device.

Example 20

Ion-Capture Flow-through Device for an hCG Assay

a. Preparation of the solid phase

A glass fiber filter material was treated with an aqueous solution of 0.125% Celquat® L-200 polymeric quaternary ammonium compound to form a positively charged capture zone. The glass fiber filter was then set upon a second layer of absorbent material which serves to pick up excess reagents and test sample which pass through the layer containing the charged detection zone.

b. Preparation of the indicator reagent

The indicator reagent was made of colloidal gold particles coated with affinity purified goat anti-β-hCG antibody. A solution containing gold chloride (100 mg) in distilled water (510 ml) was heated to boiling and mixed with 1% sodium citrate (8.0 ml). The heat was removed when the color of the solution changed from yellow to dark red (approximately three minutes). The solution was cooled to room temperature by flushing under tap water. A portion (10 ml) of the resultant gold colloid was titrated with 150 millimolar borate buffer (pH 9.0) to pH 7.0.

Fifty microliters of goat anti-β-hCG antibody (9 mg/ml) was added to the gold colloid and mixed at room temperature for one minute. The mixture was then treated with 10% bovine serum albumin (300 µl) and centrifuged at 14,000 rpm for one minute. The bottom layer of the colloid/antibody mixture (approximately 320 µl) was recovered for use as the indicator reagent.

c. Preparation of the capture reagent

Purified monoclonal anti-hCG antibodies were modified with ITC-PGA substantially in accordance with the methods described in Examples 6 and 8, above.

d. Immunoassay protocol

All reagents were appropriately diluted in an assay buffer containing 50 mM Tris, 150 mM NaCl, pH 7.5 and 3% casein. A test sample (50 µl) containing a known amount of hCG in normal human urine (0, 25, 50, 100 and 250 mIU/ml) was mixed with an equal volume of indicator reagent, and the mixture was incubated at room temperature for five minutes. Capture reagent (50 µl) was then added to the mixture. The resulting mixture was then transferred to the solid phase that had been pre-wetted with buffer (80 µl). The flow-through devices were then rinsed twice with buffer. A visible purple color was detected for those devices which received hCG-containing reaction mixtures, while the 0 mIU/ml test sample produced no detectable signal at the capture zone. The darkness of the signal at the capture zone increased with the increase of hCG concentration.

Example 21

Competitive Digoxin Assay Using Ion-Capture

a. Preparation of the solid phase

Test sample application pads (glass fiber matrix) were overcoated with various concentrations of an aqueous solution of Merquat-100® polymeric ammonium compound, 100 mM Tris, 100 mM sodium chloride, 0.1% fish gelatin, 0.1% sucrose and 0.1% sodium azide. The application pads were allowed to dry and were then overlaid upon a layer of absorbent material to prepare flow-through devices.

b. Preparation of the indicator reagent

The indicator reagent was a conjugate of digoxin dialdehyde and alkaline phosphatase, diluted in 50 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% NaN_3 and 0.1% bovine serum albumin at pH 7.5.

c. Preparation of the capture reagent

The first capture reagent, a goat anti-digoxin antibody coupled to 1,4-phenylene diisothiocyanate activated poly-L-aspartic acid (ITC-PAA), was prepared substantially in accordance with the method described in Example 8.c. above. Poly-L-aspartic acid was used in place of poly-L-glutamic acid.

A second capture reagent was made of rabbit anti-goat IgG antibody coupled to poly-L-aspartic acid using EDCI substantially in accordance with the coupling protocol described in Example 15. c, above. An analyte-specific ancillary binding member (goat anti-digoxin antibody) was used together with this capture reagent to bind the analyte to the solid phase. In one embodiment, the capture reagent was a preformed complex of the negatively charged anti-goat antibody and the goat anti-digoxin antibody. Both the first and second capture reagents were appropriately diluted before use with 50 mM Tris, 50 mM NaCl, 0.1% NaN_3 and 0.1% bovine serum albumin at pH 7.5

d. Immunoassay protocol

In assays using the first capture reagent, or direct capture system, the capture reagent (60 µl) was mixed with test sample (18 µl) containing a known amount of digoxin. The reaction mixture was incubated at approximately 33-34°C for about ten minutes. The specific binding reaction resulted in the formation of a capture reagent/analyte complex. The indicator reagent (60 µl) was then added to the reaction mixture, and the mixture was incubated for about another eleven minutes. The specific binding reaction resulted in the formation of capture reagent/indicator reagent complex in proportion to the amount of analyte present in the test sample. A portion of each reaction mixture (80 µl) was then applied to the solid phase, followed by two washes with Tris buffered saline (75 µl). An enzyme substrate (70 µl; 1.2 mM 4-methylumbelliferyl-phosphate in a solution of 100 mM AMP, 0.01% EDTA, 0.1% NaN_3, and 4.0 mM tetramisole at pH 10.3) was added, and the resulting rate of fluorescence was measured.

In an indirect assay using the second capture reagent, the preformed capture reagent/ancillary binding member complex (50 µl), indicator reagent (55 µl) and digoxin test sample (25 µl) were combined with sample diluent buffer (91 µl). The mixture was incubated for approximately nine minutes. The specific binding reaction resulted in the formation of capture reagent/ancillary binding member/analyte complex and capture reagent/ancillary binding member/indicator reagent complex in proportion to the amount of analyte present in the test sample. An aliquot of the reaction mixture (180 µl) was then applied

to the solid phase, followed by two washes with Tris buffered saline (75 µl). The enzyme substrate (70 µl) was added, and the resulting rate of fluorescence was measured.

The results of the assay are shown in Table 22. The results demonstrated that as the digoxin test sample concentration increased there was a corresponding decrease in the formation of complex containing indicator reagent. Therefore, the amount of detectable label associated with the solid phase decreased with the increase of digoxin in the test sample.

TABLE 22

Digoxin Ion-capture Competitive Assay

Protocol:	Semi-sequential One-Step	One-Step
Precoated Solid Phase:	0.2% Merquat-100®	0.2% Merquat-100®
Concentration of Antibody/Test:	162 ng Goat anti-Digoxin	90 ng Rabbit anti-Goat/ 64 ng Goat anti-Digoxin
Indicator Reagent:	Alkaline Phosphatase/Digoxin Conjugate	Alkaline Phosphatase/Digoxin Conjugate
	Direct	**Indirect**
Digoxin (ng/ml)	Rate of fluorescence (counts/sec/sec)	
0	680	456
0.5	546	387
1	413	309
2	303	247
3	261	179
5	183	121

Example 22

Ion-capture Flow-Through Device for a Total T3 (Triiodothyronine) Competitive Assay

a. Preparation of the solid phase

Test sample application pads (glass fiber matrix) were treated with various concentrations of an aqueous solution of Celquat® L-200 polymeric

quaternary ammonium compound or Merquat-100® polymeric ammonium compound, 100 mM Tris, 100 mM sodium chloride, 0.1% fish gelatin, 0.1% sucrose and 0.1% sodium azide. The application pads were allowed to dry, and the pads were overlaid upon a layer of absorbent material to form the individual assay devices.

b. Preparation of the indicator reagent

The indicator reagent was a conjugate of T3 and alkaline phosphatase, diluted in 50 mM Tris, 100 mM NaCl, 1.0 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 1.0% bovine serum albumin at pH 7.5. Dextran sulfate (MW 5,000) was included as a nonspecific binding blocker. The blocker was used to enhance the signal-to-noise ratio by inhibiting the binding of the labeled antibody to non-analyte.

c. Preparation of the capture reagent

The capture reagent, an anti-T3 antibody coupled to polyaspartic acid (PAA-anti-T3 antibody), polyacrylic acid (PAcA-anti-T3 antibody) or carboxymethyl cellulose (CMA-anti-T3 antibody) anionic polymer molecules, was prepared substantially in accordance with the method described in the Example 15. c EDCI coupling method, with the exception that no chromatographic filtration of the capture reagent was performed. The capture reagent was diluted with 800 mM Tris, 50 mM NaCl, 0.1% NaN_3, 0.01% furosemide, 0.1% Tween-20,1.0% bovine serum albumin and 0.08 mg/ml goat IgG at pH 7.4.

d. Immunoassay protocol

The capture reagent (50 µl) was mixed with an equal volume of test sample, containing a known amount of Total T3, and sample diluent buffer (150 µl). The reaction mixture was incubated for approximately 15 minutes. The specific binding reaction resulted in the formation of a capture reagent/analyte complex.

Each reaction mixture (150 µl) was then applied to a solid phase. The indicator reagent (60 µl) was then applied to the solid phase and incubated for eight minutes. The device was then washed two times. An enzyme substrate (50 µl) was added, and the resulting rate of fluorescence was measured.

In an alternative assay format, the solid phase was also washed prior to the addition of the indicator reagent. In yet another assay format, the capture reagent and test sample were combined and incubated, followed by the addition of indicator

reagent and further incubation prior to placing an aliquot of the reaction mixture on the solid phase.

The polyelectrolyte interaction of the capture reagent and the oppositely charged solid phase resulted in the immobilization of capture reagent and capture reagent complexes on the solid phase devices. An enzyme substrate (70 µl; 1.2 mM 4-methylumbelliferyl-phosphate in a solution of 100 mM AMP, 0.01% EDTA, 0.1% NaN_3, and 4.0 mM tetramisole at pH 10.3) was added, and the resulting rate of fluorescence was measured.

In each assay, the results demonstrated that as the Total T3 test sample concentration increased there was a corresponding increase in the formation of capture reagent/analyte complex, and therefore, the amount of detectable label associated with the solid phase decreased. Furthermore, the results show that the signal to noise ratio is improved by including a nonspecific binding blocker, dextran sulfate, in the indicator reagent.

TABLE 23

Total T3 Competitive Assay

Calibration data comparing one-step and two-step assay protocols

Protocol:	One-step	Two-step
Precoated Solid Phase:	0.5% Celquat®	0.2% Merquat-100®
Capture Antibody: (per test)	ITC-PGA anti-T3 antibody (0.25 µg)	EDAC-PAA anti-T3 antibody (0.02 µg)
Indicator Reagent:	no blocker	with 0.1% dextran sulfate
Calibrators concentration ng/ml total T3	Rate of fluorescence (counts/sec/sec)	
0	518	616
0.5	386	513
1.0	310	403
2.0	218	260
4.0	123	109
8.0	71	48

TABLE 24

Total T3 Competitive Two-Step Assay

comparison of indicator reagents with and without a nonspecific binding blocker

Precoated Solid Phase:	0.2% Merquat-100®	0.2% Merquat-100®
Capture Antibody: (per test)	EDAC-PAA anti-T3 antibody (0.02 µg)	EDAC-PAA anti-T3 antibody (0.02 µg)
Indicator Reagent: T3 alkaline phosphatase dilution:	no blocker 1:400	with 0.1% dextran sulfate 1:150
Calibrators concentration ng/ml total T3	Rate of fluorescence (counts/sec/sec)	
0	641	536
2.0	361	220
8.0	81	39

TABLE 25

Total T3 Competitive Assay

Calibration data comparing different T3 capture reagents

Capture Antibody: (per test)	PAA-anti-T3 antibody (0.013 µg)	PAcA-anti-T3 antibody (0.015 µg)	CMA-anti-T3 antibody (0.013 µg)
Calibrators concentration ng/ml total T3	Rate of fluorescence (counts/sec/sec)		
0	509	544	507
0.5	401	443	394
1.0	332	344	322
2.0	203	219	204
4.0	94	107	99
8.0	47	57	51

Example 23

HIV-1 Anti-p24 antibody Detection Using an Ion-Capture Sandwich Assay

The solid phase devices were prepared by overcoating glass fiber matrixes with a polycationic substance and overlaying the matrixes upon an absorbent material. The capture reagent, was prepared by the covalent coupling of a polyanionic substance to purified recombinant p24 antigen. The indicator reagent was a conjugate of alkaline phosphatase and anti-biotin antibody which bound to the analyte antibody by means of an analyte-specific ancillary specific binding member, i.e., biotinylated p24 antigen. The enzyme substrate was 4-methylumbelliferyl-phosphate.

The capture reagent was reacted with the test sample to form a capture reagent/analyte complex. Excess reagent and test sample components were removed and the complex was immobilized by passage through the oppositely charged solid phase. The amount of captured analyte was then determined by the sequential addition of the ancillary specific binding member, indicator reagent and enzyme substrate.

Example 24

Ion-Capture Device with Procedural Control

In an alternative embodiment, the solid phase reaction matrix of Example 17 was prepared such that two assay reagents were incorporated into the matrix in an overlapping design to form the detection zone. The reaction of one reagent completed one portion of a detectable pattern, and the reaction of a second reagent completed another portion of the detectable pattern.

For example, the anionic polymer (such as polyglutamic acid) was applied to the solid phase to form the vertical bar of a "cross" shaped design. The anionic polymer attracted and attached to the oppositely charged capture reagent comprising an analyte-specific binding member conjugated to a polymeric cation. The reaction of the capture reagent, analyte and an indicator reagent specific for the analyte resulted in a detectable complex being immobilized at the vertical bar.

A procedural control reaction zone, which did not involve an analyte reaction, was formed in the shape of the horizontal bar of the cross-shaped detection zone. A reagent which reacted with and immobilized the indicator reagent without the formation of an analyte-containing complex was used.

For example, when the indicator reagent was made of colloidal gold particles coated with affinity purified goat anti-β–hCG antibody, then the horizontal bar of the cross-shaped detection zone included a specific binding member which would directly bind to the goat anti-β–hCG antibody, e.g., a rabbit anti-goat antibody. Thus, detectable label was immobilized in the horizontal bar whether or not there was analyte present in the test sample.

It will be appreciated by one skilled-in-the-art that the concepts of the present invention are equally applicable to any separation techniques or homogeneous binding assays (wherein the signal generating ability of the label is not altered during the binding reaction) by using oppositely charged solid phase materials and capture reagents. The embodiments described in detail herein are intended as examples rather than as limitations of the polyelectrolyte reactions and assays. Thus, the description of the invention is not intended to limit the invention to the particular embodiments described, but it is intended to encompass all equivalents and subject matter within the spirit and scope of the invention as described above and as set forth in the following claims.

CLAIMS

What is claimed is:

1. A method for determining the presence or amount of an analyte in a test sample, comprising the steps of:

a) providing

(i) a capture reagent, comprising a first binding member conjugated to a polymeric anion substance,

(ii) an indicator reagent, comprising a second binding member and a detectable label, and

(iii) a solid phase material containing a reaction site comprising a polymeric cation substance having a nitrogen content of at least about two percent;

wherein said first and second specific binding members are selected from binding members capable of forming a complex with the analyte in a sandwich assay, a competitive assay or an indirect assay, and thereby forming a detectable complex in proportion to the presence or amount of the analyte in the test sample;

b) contacting said solid phase with said capture reagent and the test sample, whereby said polymeric cation of said solid phase attracts and attaches to said polymeric anion of said capture reagent, thereby immobilizing said capture reagent and complexes thereof;

c) contacting said solid phase with said indicator reagent, whereby said indicator reagent becomes bound to said immobilized capture reagent or complex thereof in proportion to the amount of analyte present in the test sample; and

d) detecting said indicator reagent associated with said solid phase to determine the presence or amount of the analyte in the test sample.

2. The method according to Claim 1, wherein said polymeric cation substance is a polymeric quaternary ammonium compound having a nitrogen content of at least about two percent.

3. The method according to Claim 1, wherein said polymeric cation substance has a nitrogen content of at least about five percent.

4. The method according to Claim 1, wherein said polymeric cation substance has a nitrogen content of at least about ten percent.

5. The method according to Claim 1, wherein said polymeric cation substance is incorporated in or on said solid phase immediately before performance of the assay process.

6. The method according to Claim 1, further comprising the step of combining the test sample and said capture reagent to form a reaction mixture, wherein said reaction mixture is contacted to said solid phase.

7. The method according to Claim 1, further comprising the addition of an ancillary specific binding member, wherein said ancillary specific binding member is capable of binding the analyte and is capable of binding a member selected from the group consisting of said indicator reagent or said capture reagent in an indirect assay.

8. A method for determining the presence or amount of an analyte in a test sample, comprising the steps of:

a) providing

(i) a capture reagent, comprising a first binding member conjugated to a polymeric anion substance,

(ii) an indicator reagent, comprising a second binding member and a detectable label,

(iii) a non-specific binding blocker comprising an unbound polymeric anion material, and

(iv) a solid phase material containing a reaction site comprising a polymeric cation substance having a nitrogen content of at least about two percent;

wherein said non-specific binding blocker inhibits the non-specific binding of reagents to said solid phase, and wherein said first and second specific binding members are selected from binding members capable of forming a complex with the analyte in a sandwich assay, a competitive assay or an indirect assay, and thereby forming a detectable complex in proportion to the presence or amount of the analyte in the test sample;

b) contacting said solid phase with said capture reagent and the test sample, whereby said polymeric cation of said solid phase attracts and attaches

to said polymeric anion of said capture reagent, thereby immobilizing said capture reagent and complexes thereof;

c) washing said solid phase;

d) contacting said solid phase with said indicator reagent, whereby said indicator reagent becomes bound to said immobilized capture reagent or complex thereof in proportion to the amount of analyte present in the test sample; and

e) detecting said indicator reagent associated with said solid phase to determine the presence or amount of the analyte in the test sample.

9. The method according to Claim 8, further comprising the step of combining the test sample and said capture reagent to form a reaction mixture, wherein said reaction mixture is contacted to said solid phase.

10. The method according to Claim 8, further comprising the addition of an ancillary specific binding member, wherein said ancillary specific binding member is capable of binding the analyte and is capable of binding a member selected from the group consisting of said indicator reagent or said capture reagent in an indirect assay.

INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US92/02980

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,780,409 (Monji et al) 25 October 1988, col. 2, lines 38-46; col. 3, lines 39-64; col. 6, lines 44 and 66; col. 8, lines 36-62; col. 9, lines 47-51; col. 10, lines 30-57; col. 11, line 29 - col. 12, line 31.	1-10
Y	US, A, 4,935,147 (Ullman et al) 19 June 1990, col. 1, lines 29-40; col. 3, lines 5-15; col. 4, lines 4-35; col. 7, lines 25-27 and 54-55; col. 8, lines 6-9; col. 10, line 65 - col. 12, line 3; col. 13, lines 37 and 53; col. 15, lines 15 and 37-40; col. 17, lines 13-14; col. 18, lines 48-64.	1-10
Y,P	US, A, 5,094,962 (Snyder et al) 10 March 1992, col. 3, lines 11-20; col. 4, line 66 - col. 5, line 49; col. 14, lines 37-43; and Example 4.	1-10
Y	US, A, 4,121,975 (Ullman et al) 24 October 1978, col. 1, lines 50 and 61-68.	8-9
Y,P	US, A, 5,051,356 (Warren, III et al) 24 September 1991, abstract and col. 4, line 60 - col. 5, line 32.	8-9
Y	US, A, 4,530,900 (Marshall) 23 July 1985, col. 1, line 55 - col. 3, line 36.	7, 10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 03 AUGUST 1992	Date of mailing of the international search report 10 AUG 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CAROL A. SPIEGEL Telephone No. (703) 308-0196

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INTERN.' 'ONAL SEARCH REPORT

Ir ational application No.
PCT/US92/02980

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C12Q 1/00, 1/68; G01N 33/53, 33/536, 33/537, 33/538, 33/541, 33/543, 33/544, 33/546, 33/551, 33/553; C11D 3/07, 3/066

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 7.9, 7.92, 7.93, 7.94, 7.95; 436/111, 518, 523, 524, 525, 526, 530, 534, 535, 536, 538, 540, 541; 252/528; 428/402, 402.22, 407

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

435/6, 7.1, 7.9, 7.92, 7.93, 7.94, 7.95, 805, 810, 970, 971, 975; 436/111, 518, 523, 524, 525, 526, 530, 534, 535, 536, 538, 540, 541, 808, 810, 824, 825; 252/528; 428/402, 402.22, 407

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS,
search terms: hexadimethrine bromide, polybrene, quaternary ammonium, gafquat, diethylaminoethyl dextran, dimethylallylammonium, immunoassay, salt, ion, ionic